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TITLE: The Role of the Phosphatidylinositol-5-Phosphate 4-Kinases in p53-Null Breast Cancers

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14. ABSTRACT I have identified a family of druggable enzymes whose loss of function results in synthetic lethality with p53 loss. Prior to my observation of this synthetic lethality, these enzymes were not a focus for oncology research. My preclinical studies in genetically engineered mouse models and in human breast cancer cell lines and xenografts indicate complete inhibition of cell growth upon loss of these enzymes in the context of p53 mutation or deletion, but no effect on growth in cells containing functional p53. As I mentioned above through an R03 grant we have screened a library of drug-like molecules and identified a number of highly specific inhibitors of PI5P4K. Additionally, through the collaboration with Dr. Nathanael Gray's laboratory (Dana Farber Cancer Institute) to develop covalent inhibitors of PI5P4K we have demonstrated compounds that cause >95% inhibition of PI5P4K. These compounds provide me with tools for interrogating the mechanism of synthetic lethality and for exploring <i>in vivo</i> efficacy/toxicity in my mouse models. With all these tools in hand I am well equipped to tackle key questions surrounding the role of these essential enzymes not only in cancer biology but in normal biological processes.					
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## 1. INTRODUCTION:

The goal of this Project is to evaluate the role of a novel class of phosphoinositide kinases, the phosphatidylinositol-5-phosphate 4-kinases (PI5P4Ks) in the growth of breast cancers. My recent studies, based on gene deletion in mice, reveal that these enzymes control glucose homeostasis, insulin sensitivity and susceptibility to tumor formation in p53 mutant backgrounds, raising the possibility that inhibitors of these 'druggable' enzymes could be efficacious in treating breast cancer, in particular p53 null breast cancers. My application proposes to understand the biochemical basis for the function of these enzymes and to further validate them as targets for pharmaceutical intervention in breast cancer.

The most frequently mutated gene across all types of cancers is a gene called p53.

Unfortunately it has been difficult to directly target this gene with drugs. I have identified a family of enzymes that is crucial for the growth of cancers that have genetic aberrations in p53. Targeting these enzymes with novel agents might prevent the growth of p53 mutant cancers, thereby benefiting a broad spectrum of cancer patients, including those with breast, ovarian, lung, colorectal and brain tumors. The Type 2 phosphatidylinositol-5-phosphate 4-kinases  $\alpha$  and  $\beta$  (Type 2 PIP kinases) are essential for cancer growth when cells have lost p53, the powerful tumor-suppressor gene long dubbed the "guardian of the genome." More than half of all cancers lose this gene, allowing these cancers to grow at will. I have discovered that the Type 2 PIP kinases are not critical for the growth of normal cells but become essential for cell growth when p53 is lost due to mutations or deletions. The fact that we can delete the Type 2 PIP kinases in normal human cells or in mice with essentially no effect on cell survival suggests that inhibitors of these enzymes should have little toxicity in patients. Additionally, a subset of breast cancers express high levels of these molecules; therefore I looked at their role in HER2-positive breast cancers, which typically are more aggressive tumors. Interestingly, I found a strong correlation with the amplification *PIP4K2B* in HER2-positive breast cancers as well as a co-occurrence with *TP53* mutations or deletions. My research proposed in this application will further characterize the role of these enzymes in breast cancer and will give rise to concrete evidence that these very 'druggable' enzymes would be excellent targets for breast cancer treatment, ultimately having a significant impact on patient care.

## 2. KEYWORDS:

p53  
phosphoinositide kinases  
breast cancer  
autophagy  
metabolism  
synthetic lethal

## 3. ACCOMPLISHMENTS:

### What were the major goals of the project?

The **first goal** of this project is to provide proof-of-concept that targeting *PIP4K2A* and/or *PIP4K2B* would be an effective therapy for *TP53* mutant breast cancers. A **second goal** is to develop novel breast cancer mouse models in order to determine the impact of PI5P4K

suppression/deletion on tumorigenesis in mouse models of breast cancer. The **third goal** is to decipher the biochemical mechanism(s) by which loss of PI5P4Ks prevents breast cancer.

#### **What was accomplished under these goals?**

To date I have shown that breast cancer cell lines with wild type *TP53* are unaffected by knockdown of PIP4K2A and B, while *TP53* mutant cell lines stop growing or die when both PIP4K2A and B are knocked down with shRNA. I have now obtained relatively specific inhibitors of PIP4K2A and B (KIs of 10-200 nM) through an R03-funded screen at NCATS. In addition, we have developed covalent inhibitors of PIP4K2A and B through collaboration with Dr. Nathanael Gray (Dana Farber Cancer Institute). These inhibitors mimic the shRNA knockdown, showing no effect on p53 wild type cells and inhibition of growth or induction of death in p53 mutant cells. I am currently utilizing both shRNA and inhibitors to screen a larger panel of breast cancer cell lines and determine the subset of cell lines that are most sensitive to inhibition or knockdown of PIP4K2A/B and correlate this with genetic aberrations (e.g., gene amplifications of PIP4K2A or PIP4K2B, or mutations in *TP53*). To date I have screened approximately 30 breast cancer cell lines. As stated in the SOW I am on target and I am efficiently achieving my milestones that were set for year one. Additionally, we are using CRISPR technology to generate isogenic *TP53* wild-type versus *TP53* deleted breast cell lines and are generating CRISPR/Cas9 constructs for knocking out both PIP4K2A and PIP4K2B. This approach will provide a complete deletion and should be more effective than shRNA in eliminating the two proteins in the breast cancer cell lines. Furthermore, we have optimized IHC protocols for both PIP4K2A and PIP4K2B and are currently screening large cohorts of breast tissue microarrays and will correlate the expression of the kinases with patient data.

Currently, we are making tremendous progress on the development of our extremely complex mouse models in order to study the genetic basis of cancer and the therapeutic potential of targeting the lipid kinases, PI5P4Ks. We are using a variety of breast mouse models with defined genetic mutations to understand how these mutations contribute to the development and progression of breast cancer. We are still in the breeding phase and generating all the necessary mouse lines in order to perform our experiments. I am optimistic that by the end of 2016 we will begin to follow tumor development in these mice to determine whether homozygous deletion of *PIP4K2B* alone is sufficient to suppress tumor growth in the context of breast-specific *TP53* deletion or whether both *PIP4K2A* and *B* must be deleted to suppress growth. As far as milestones for the development of these complex cancer models we are on target.

In my attempt to map out the molecular mechanisms by which the inhibition of PI5P4K specifically impairs the growth and survival of *TP53* mutant breast cancer cells and not *TP53* wild type cells and I have discovered that the PI5P4K enzymes are required for autophagy. I have found that suppression of both PIP4K2A and B activity with shRNA or small molecule inhibitors causes accumulation of cleaved LC3 and a dramatic build up of autophagic vesicles in *TP53* deficient cells but that these same agents have no effect on the level of cleaved LC3 or on autophagic vesicles in *TP53* wild type cells. These effects on *TP53* mutant cells correlate with decreased glycolysis and decreased oxidative phosphorylation and cell stasis or cell death. I am currently using shRNA, siRNA, inducible knockdowns and small molecule inhibitors to tease out

the biochemical pathway by which suppression of PIP4K2A/B results in defective autophagy and impaired cell metabolism. I am extremely excited about these recent findings and I am on target with defining the role of the PI5P4K enzymes in maintaining glucose metabolism and why they are only be critical in the context of p53 loss.

**What opportunities for training and professional development has the project provided?**

Nothing to Report

**How were the results disseminated to communities of interest?**

Nothing to Report

**What do you plan to do during the next reporting period to accomplish the goals?**

Thus far, I am finding that loss of function of PIP4K2A and B is selectively resulting in cell stasis or cell death in breast cancer cell lines that have loss of function of *TP53*, either deletion or mutations. The expectation is that the covalent and non-covalent inhibitors of PIP4K2A and B will mimic the double knockdown of these enzymes in regard to which cell lines respond and which are resistant. If these results come out as expected, I will have considerable confidence that *TP53* mutant breast cancer cell lines require either PIP4K2A or PIP4K2B protein for growth. The sensitivity to the inhibitors also indicates that they require the catalytic activity of these enzymes and will provide some validation that small molecule inhibitors of PIP4K2A and B could be effective therapies for *TP53* mutant breast cancers. During the next reporting period I hope utilize both shRNA and inhibitors to screen a large panel of breast cancer cell lines and human 3D breast cancer organoids developed at our Institute of Precision Medicine in order to to discover other genetic events (in addition to *TP53* mutations) or gene expression signatures across the cell lines that correlate with sensitivity to knockdown or acute inhibition of PIP4K2A and B in regard to cell growth. At the conclusion of the next report period, I anticipate understanding the constellation of genomic alterations most associated with response to PI5P4K modulation.

In addition to the *in vitro* experiments above the mouse models are coming along and for the next reporting period I anticipate having data to address the role of PI5P4K *in vivo* using the mouse models of breast cancer that I have developed. Additionally, in collaboration with Weill Cornell Cancer Center I currently have 10 breast cancer patient derived xenograft (PDX) models growing. These models can be ranked according to differential response to therapy, to discover potential biomarkers that predict the subset that respond to PIP4K2A/B suppression. While waiting for our mouse models to be generated I plan to will utilize siRNAs targeting PIP4K2A and B as well as out novel PI5P4K inhibitors in these PDX models. With these models I will really be able to tease out whether the PI5P4K enzymes are essential in p53 deleted or p53 mutated breast cancers, providing a clear genetic evaluation of PI5P4K ablation in an *in vivo* setting.

Finally, to examine my hypothesis that the PI5P4K are required for autophagy in TP53 mutant breast cancer cells I will use my genetic mouse models and cell lines that I have developed as well as characterize the effects of PI5P4K shRNAs and novel PI5P4K inhibitors on autophagy flux

and metabolism in a panel of breast cancer cell lines. Furthermore, as stated above using CRISPR technology I plan to generate isogenic *TP53* wild-type versus *TP53* deleted breast cancer cell lines.

#### **4. IMPACT:**

##### **What was the impact on the development of the principal discipline(s) of the project?**

Nothing to Report

##### **What was the impact on other disciplines?**

Nothing to Report

##### **What was the impact on technology transfer?**

During the first year of this award we have been very successful and I will highlight three major impacts that have been made. Furthermore, these innovations have led to the excitement of many well-established pharmaceutical companies and new start-up companies targeting the PI5P4K enzymes for p53 mutant cancers.

1) We have identified a family of druggable enzymes (PIP4K2A/B) whose loss of function results in synthetic lethality with p53 loss. Prior to our observation of this synthetic lethality, these enzymes were not a focus for oncology research. Our preclinical studies in genetically engineered mouse models and in human breast cancer cell lines and xenografts indicate complete inhibition of cell growth upon loss of these enzymes in the context of p53 mutation or deletion, but no effect on growth in cells containing functional p53.

2) Through an R03 grant we have screened a library of drug-like molecules and identified a number of highly specific inhibitors of PIP4K2A and B (see letter of collaboration from Anton Simeonov). These inhibitors are in the 10 to 500 nM range. We have shown that some of these inhibitors efficiently enter cells and mimic the effects seen with knockdown of PIP4K2A and B: complete inhibition of growth of *TP53* mutant cells, with no effect on *TP53* wild type cells. We have also collaborated with Nathanael Gray's laboratory (Dana Farber Cancer Institute) to develop covalent inhibitors of PIP4K2A and B and demonstrated that these compounds cause >95% inhibition of both PIP4K2A and B within 4 hours of treatment at 1 micromolar concentration. These compounds also completely parallel the effects of the shRNA knockdown experiments (complete inhibition of growth of *TP53* mutant cells, with no effect on *TP53* wild type cells – see below). The covalent inhibitors are based on a scaffold similar to that used for the drug gleevec and are likely to have good PK/PD. These compounds provide us with tools for interrogating the mechanism of synthetic lethality and for exploring *in vivo* efficacy/toxicity in our mouse models in the Pre-clinical models and therapeutics Core.

3) While my preliminary results suggest that PIP4K2A/B play a role in lysosome and autophagosome function, inhibition of these targets results in very different consequences from inhibition of other targets that are known to affect autophagy, and they have no effect on cells that are wild type for *TP53*, suggesting a novel role for these enzymes in *TP53* mutant tumors and a better efficacy/toxicity window than other drugs developed to target autophagy.

##### **What was the impact on society beyond science and technology?**

Nothing to Report

#### 4. CHANGES/PROBLEMS:

##### Changes in approach and reasons for change

No significant changes have been made during this reporting period. However, in generating the mouse models we have decided to focus on a couple of key models and have developed PDXs that will be extremely useful in the pursuit of this grant.

I have now generated the  $PIP4K2A^{flx/flx} PIP4K2B^{-/-} TP53^{flx/flx}$  and  $PIP4K2B^{-/-} TP53^{flx/flx}$  mice and I am crossing these mice to the both the K14-Cre  $BRAC1^{flx/flx} TP53^{flx/flx}$  mouse model, which develop triple negative basal breast cancers rapidly (~3 months), and the MMTV-Cre HER2/neu transgenic mouse model that develop mammary carcinomas with 100% penetrance by six months of age. In addition to these models, I have also crossed out the Cre in order to initiate breast carcinoma by intraductal injections of adenoviral Cre. I have chosen to do this because the K14 Cre animals tend to get mild to severe skin infections and the MMTV Cre tends to be leaky, thereby confounding the results. Additionally, I have obtained the p53 gain of function (hot-spot mutation) conditional mouse models, R172H (R175H in human) and R270H (R273H in humans) from Dr. Ken Olive (Olive et. al, 2004), which I plan to cross with my  $PIP4K2A^{flx/flx} PIP4K2B^{-/-}$  mice. With these models I will really be able to tease out whether the PI5P4K enzymes are essential in p53 deleted or p53 mutated breast cancers, providing a clear genetic evaluation of PI5P4K ablation in an *in vivo* setting. Another exciting model I am working on in collaboration with Dr. Scott Lowe's laboratory (MSKCC) is an inducible shRNA mouse targeting both  $PIP4K2A$  and  $PIP4K2B$  in order to globally knockout the kinases in the mouse. This approach will allow me to quickly generate mice with breast specific deletions of  $TP53$  and  $BRCA1$  with the ability to globally turn off  $PIP4K2A$  and  $B$  in a reversible manner after the breast tumors are established. Since this approach is moving very quickly, it is likely that I will already have data in place by the next reporting period.

$BRCA1$  with the ability to globally turn off  $PIP4K2A$  and  $B$  in a reversible manner after the breast tumors are established.

##### Actual or anticipated problems or delays and actions or plans to resolve them

No significant problems anticipated

##### Changes that had a significant impact on expenditures

Nothing to report

##### Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

As described above I have chosen to focus on a couple of key breast cancer models and now have PDX models. Additionally, I anticipate that by the end of next reporting period we will have the inducible shRNA mouse targeting both  $PIP4K2A$  and  $PIP4K2B$  in order to globally knockout the kinases in the mouse.

#### 5. PRODUCTS:

- Publications, conference papers, and presentations

## **SELECTED PRESENTATIONS**

- **Emerling BM**, Hurov JB, Poulogiannis G, Tsukazawa KS, Wulf G, Bell EL, Shim H, Choo-Wing R, Bellinger G, Lamia KA, Rameh LE, Sasaki A, Asara JM, Yuan X, Bullock A, Brown V, Signoretti S, and Cantley LC. Deletion of Phosphatidylinositol-5-phosphate 4-Kinases results in impaired growth of *TP53*<sup>-/-</sup> tumors. *Annual AACR Meeting: Major Symposium Synthetic Lethality (2014)*
- **Emerling BM**, Hurov JB, Poulogiannis G, Tsukazawa KS, Wulf G, Bell EL, Shim H, Choo-Wing R, Bellinger G, Lamia KA, Rameh LE, Sasaki A, Asara JM, Yuan X, Bullock A, Brown V, Signoretti S, and Cantley LC. Deletion of Phosphatidylinositol-5-phosphate 4-Kinases results in impaired growth of p53 deficient tumors by mediating changes in metabolism. *Beatson International Cancer Conference: Powering the Cancer Machine (2014)*
- **Emerling BM**, Yang Z, Loughran R, Yang TJ, Johnson J, Pragani R, Davis M, Hu X, Shen M, Boxer M, Simeonov A and Cantley LC. Targeting p53 mutant cancers through inhibition of the Phosphatidylinositol-5-Phosphate 4-Kinases. *Annual AACR Meeting: MiniSymposium Targeting Signaling Pathways in Cancer (2015)*
- **Technologies or techniques**

### **Development of novel PI5P4K inhibitors for cancer therapy**

As I mentioned above through an R03 grant we have screened a library of drug-like molecules and identified a number of highly specific inhibitors of PI5P4K. Additionally, through the collaboration with Dr. Nathanael Gray's laboratory (Dana Farber Cancer Institute) to develop covalent inhibitors of PI5P4K we have demonstrated compounds that cause >95% inhibition of PI5P4K. These compounds provide me with tools for interrogating the mechanism of synthetic lethality and for exploring *in vivo* efficacy/toxicity in my mouse models. With all these tools in hand I am well equipped to tackle key questions surrounding the role of these essential enzymes not only in cancer biology but in normal biological processes.

- **Inventions, patent applications, and/or licenses**

Currently, we are completing the process of our patent application (Application No.: 13/985,462): MODULATION OF PHOSPHATIDYLINOSITOL-5-PHOSPHATE-4-KINASE ACTIVITY

## **6. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:**

**What individuals have worked on the project?**

<b>Name:</b>	Brooke Emerling
<b>Project Role:</b>	PI
<b>Researcher Identifier (e.g. ORCID ID):</b>	N/A
<b>Nearest person month worked:</b>	12

**Contribution to Project:** Dr. Emerling designs, executes and coordinates all experiments proposed in this project. She is responsible for data analysis and writes and edits manuscripts and research reports that emerge from this research, prepares progress reports, and presents findings at professional conferences.

**Funding Support:** The Mary Kay Foundation

**Name:** Zhiwei Yang  
**Project Role:** mouse technician  
**Researcher Identifier (e.g. ORCID ID):** N/A  
**Nearest person month worked:** 5

**Contribution to Project:** Mr. Yang is responsible for performing all implant surgeries and tumor biopsies, treating tumor-bearing animals, and maintaining the mouse colonies, setting up breeding, typing and tumor assessment.

**Funding Support:** The Mary Kay Foundation

**Name:** Ryan Loughran  
**Project Role:** research technician  
**Researcher Identifier (e.g. ORCID ID):** N/A  
**Nearest person month worked:** 5

**Contribution to Project:** Mr. Loughran works closely with Dr. Emerling and the Mr. Yang to perform the proposed experiments, to treat tumor-bearing animals and documenting and analyzing treatment outcomes.

**Funding Support:** No change

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

I have recently been awarded the Mary Kay Foundation Innovative Translational Grant Award. The goal of this project will be to investigate the role of PI5P4K in HER2+ breast cancers and I am the PI.

**What other organizations were involved as partners?**

Nothing

**7. SPECIAL REPORTING REQUIREMENTS:**

None

**8. APPENDICES:**

None